Development of a Pupal Color-Based Genetic Sexing Strain of the Melon Fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae)

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ABSTRACT The first genetic sexing system for the melon fly, *Bactrocera cucurbitae* (Coquillett), based on pupal color was developed. The recessive white pupae mutant, *wh*, was used in a putative chromosome translocation linking the wildtype allele to the male sex. This system permits the separation of males (wildtype brown pupae) from females (mutant white pupae). Results from laboratory studies indicated that egg hatch averaged 42% for flies 2–5 wk old, with higher rates (≈50%) for younger flies in this age range. Male larvae left the diet to pupate earlier than females, averaging >60% of the pupae in the first day of collection, whereas only ≈40% on the fourth day of collection. Adult emergence rate was normal, averaging ≈92%. Adult egg fecundity and fertility (both sexes) were very low (<1% of normal) after irradiation at 100 Gy at 1, 2, or 3 d before emergence. Adult flight ability from standard 20-cm tubes averaged ≈65%. Irradiated females landed on, and oviposited into, zucchini fruit significantly fewer times than nonirradiated females. Field cage survival tests indicated that the new strain survived as well (>80%) as wild males over a 7-d period when provided with food and water. Mating tests indicated that male-only sterile flies mated significantly more (about double) with wild females than sterile males from bisexual (male and female) sterile populations competing with wild males. Males fed only sugar until tested in the cages failed to mate with wild females, whereas males fed a low protein diet (6:1 sugar:protein) or the standard 3:1 diet mated as well as wild males for wild females. Finally, males exposed to the attractant, cuelure, failed to improve their mating performance compared with control, unexposed males. The results are discussed in the context of the potential for using the new strain in current or future melon fly sterile insect technique programs in Hawaii and elsewhere.

KEY WORDS genetic sexing, sterile insect technique, quality control, melon fly, *Bactrocera*

THE MELON FLY, *Bactrocera cucurbitae* (Coquillett), is a serious economic pest of fruits and vegetables, especially cucurbits, in Asia and the Pacific (White and Elston-Harris 1992). Control of tephritid pests has traditionally been carried out by chemical means, such as protein bait sprays or male annihilation (Steiner et al. 1965), but more recently, biological control techniques, especially the sterile insect technique (SIT), have gained wider use because of their environmentally benign nature. Large-scale control or eradication programs using the SIT against the melon fly were successful in the Pacific basin, especially in Japan, where the melon fly was eradicated (Kakinohana et al. 1990, Hibino and Iwahashi 1991). Many SIT programs around the world have been initiated in recent decades against the related tephritid pest, the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). One important factor in promoting the use of the SIT against *C. capitata* has been the development of effective genetic sexing strains that permit the separation of males from females at some developmental stage (Robinson et al. 1999). By releasing only sterile males, the efficiency of the technique can be increased several-fold, and fruit damage caused by stings of sterile females is avoided (McInnis et al. 1994, Rendon et al. 2000).

A genetic sexing system for the melon fly was recently developed, based on a mutant, bubble wing (*bw*), in which female adults are flightless, whereas male adults are unaffected (McCombs et al. 1993). A more effective sexing strain would be one in which the sexes are separated at an earlier stage of development, e.g., the pupal stage, or optimally, at the egg stage. Such sexing systems based on pupal coloration have been developed for two other tephritids, *C. capitata* (Robinson and van Heemert 1982) and the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (McCombs and Saul 1995). In each of these cases, chromosomal translocations link the gene for normal brown pupae color to the male sex (Y chromosome), resulting in brown pupae males, whereas females have the mutant white pupal color. Using this sexing system, photoelectric sorting machines are used to separate males from females at high speed to obtain the large numbers of

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males for release in SIT programs (e.g., McInnis et al. 1994).

In this study, we report the discovery and development of the first pupal color-based sexing system for the melon fly. After its discovery, a series of quality control studies was completed under both laboratory and field conditions. These studies were designed to evaluate the suitability of the new strain for mass production and release in melon fly SIT programs.

Materials and Methods

Origin of the Sexing Strain. In 2001, we induced a desirable chromosomal translocation through low-dose gamma irradiation. Melon flies used in this study were reared at the USDA/ARS/PBARC Laboratory in Honolulu, HI. Approximately 100 male flies (2–4 h old) were placed in a plastic cup and irradiated in air at the University of Hawaii's Cobalt ^{60} pool-type irradiator at a dose of 3.5 Kr (35 Grays). From over 300 individual F1 families, a single family was isolated that had the characteristics of a genetic sexing strain. Several hundred additional males were screened without success.

A white puparium mutant (*wh*) was isolated by D. O. M. several years ago from the standard melon fly strain reared at the USDA laboratory (McCombs et al. 1996). Genetic studies confirmed it to be a single autosomal recessive mutation located on chromosome C (McCombs et al. 1996). The puparium color is very similar to that of white pupae mutants found in oriental and Mediterranean fruit flies (Rossler 1979, McCombs and Saul 1992).

After irradiation, groups of 100 wildtype males were mass-mated to ≈100 virgin white pupae mutant females, and individual F1 males (350 total) were crossed with two virgin *wh* females in small containers. The F2 progeny of one male yielded 14 pupae—7 brown and 7 white. Of these, five males (zero females) emerged from brown pupae, whereas six females (zero males) emerged from white pupae. These individuals were inbred, and the F3 progeny confirmed the sex difference in pupal color, i.e., 127 males (0 females) emerged from brown pupae, and 135 females (0 males) emerged from white pupae. This new pupal color line was termed the T1 (first translocation) sexing strain.

Fly Rearing Parameters. The T1 strain was reared in the quarantine laboratory at the USDA/ARS fruit fly facility in Honolulu, HI. The strain was expanded as rapidly as possible after its discovery, resulting, in a few months, in the regular production of thousands of individuals per sex. Environmental conditions were 25 ± 2°C, 50–70% RH, and 10:14 L:D, with natural light supplied by a large window. Pure stocks were maintained by hand sorting up to ≈10,000 pupae per color per generation and documenting, then discarding, any rare contaminant flies (white pupae males and brown pupae females). Adult flies were maintained on food consisting of sugar (sucrose) and hydrolyzed yeast protein (3:1 by volume) and water inside cubical cages (25 cm on a side) holding ≈200 flies per sex. Eggs were collected from flies 2–6 wk old for 12–24 h in plastic cups perforated with holes containing a sponge moistened with water. Eggs were seeded onto blotter paper and placed on dishes containing 400 g of larval diet. For each generation, samples of 100–200 eggs were taken for egg eclosion rates and set on blotter paper alongside the main collection of eggs (2,000–4,000) on larval diet. The larval diet was the standard wheat-based melon fly diet based closely on the diet for the Mediterranean and oriental fruit flies (Tanaka et al. 1969). Diet dishes were placed inside fiberglass boxes containing vermiculite as a pupation medium. After larval development, pupae were collected by sifting the vermiculite with hand strainers. Pupal color differences could be seen after ≈ 2 d at ambient temperatures in vermiculite. Samples of some pupal collections were hand sorted by color, and total volume was recorded for each day of pupation. Pupae could be divided into the large classes of brown or white pupae, plus the small classes of gray (dying) or black (dead) pupae (usually <5% of total pupae).

Samples of pupae (several hundred or ≈10 ml) were collected for scoring the percentage of fly emergence and the proportion per sex for each pupal color. The level of phenotypic contamination or genetic recombination based on pupal color, i.e., females from brown pupae and males from white pupae, was also determined from brown and white pupae samples for each of 4 d of pupation (virtually all pupation occurred over 3 d). A stock colony of 5,000–20,000 phenotypically pure individuals (brown pupae males, white pupae females) was maintained continuously. Virgin flies were scored in a cold room (5°C) at 1–2 d old and mixed, phenotypically pure, at a rate of 150 males/250 females into cubical cages. Phenotypically contaminant individuals were test crossed (to white pupae stock flies) to determine if they were true genetic recombinants or simply environmentally induced color variants (e.g., nutritionally deficient). During the first five generations after the formation of the new strain (F3 generation), the following rearing data were gathered: percent egg hatch, percent pupal yield, percent brown and white pupae, percent genetic contamination, percent adult emergence, and percent flight ability.

After 6 mo of small-scale rearing, we began larger-scale tests that involved setting 6–8 ml of eggs (≈100,000 eggs) onto 5 liters of standard larval diet for melon flies (Tanaka et al. 1982). This egg volume is 50–100% higher than for a normal strain to compensate for the lower egg fertility of the T1 strain.

Laboratory Quality Control Tests. The following tests were performed to assess the quality of the T1 strain in the laboratory: flight ability tests, oviposition damage by sterile females, and effect of fly age at time of irradiation on egg fecundity and fertility. Flight ability was measured using the standard procedure adopted in most tephritid rearing facilities worldwide (Food and Agriculture Organization/International Atomic Energy Agency/U.S. Department of Agriculture 1998), except that a longer flight tube (20 cm instead of 10 cm) was used. Emerging flies were al-

lowed to fly out of the black tubes coated with talcum powder into a large adult holding cage, and the pupae were scored for percent emergence and percent fliers after adults finished emerging over a 3-d period. Ten replicate tubes were run from pupae collected on the major larval collection day (usually the second collection day). Five of these tubes were set up with pupae held under ambient conditions, whereas another five tubes were set up with pupae held in the cooler standard pupal holding room at 20°C for 12 d after sifting from vermiculite at 2 d of age. Seven replications were completed between May and July 2002.

Tests were initiated to evaluate the effect of variable pupal age at the time of irradiation on egg fertility. For these studies, a Gammacell 220 (MDS Nordion, Kanata, Ontario, Canada) gamma irradiator was used at a dose of 100 Gy, a standard dose for melon fly SIT programs. This dose had recently been tested on the T1 melon fly strain and the standard laboratory bisexual strain, producing reliably high egg sterility (>99.5%) on random samples of pupae irradiated at an average target pupal physiological age of −2 d (i.e., 2 d before peak adult emergence). Pupae were irradiated at particular developmental ages (−1, −2, and −3), emerging adults were separated by sex daily, and the following 10 mating crosses were made (50♂ × 50♀): Normal (N; nonirradiated) × N, N × −1, −1 × N, −1 × −1, N × −2, −2 × N, −2 × −2, N × −3, −3 × N, and −3 × −3. Flies were held under ambient conditions in cubical cages with standard food (3:1 sugar: protein) and water. Adult T1 flies mature sexually at ≈10 d of age, so we chose to allow fly oviposition at this age (1.5 wk) and again at 2.5 wk of age. Eggs were collected in plastic cups, and up to 2,000 eggs were streaked onto moist blotter paper in a Petri dish to allow egg hatch. Three days later the numbers of eggs and egg hatch rates were scored for each treatment.

The final laboratory fly quality study evaluated host fruit damage from oviposition by normal or irradiated T1 females in the laboratory. Such a study is needed to determine the potential impact of released sterile females, even if very rare among all released flies. Females were mixed with males of the same type (normal or irradiated) and removed from their cages for testing at 1.5, 2.5, or 3.5 wk of age. The oviposition tests were conducted under the same ambient conditions as above in a large (0.6 by 0.4 by 0.4 m) transparent plastic cage. A ripe zucchini fruit was hung from the ceiling of the cage, and 50 females of a given age were released into the cage and observed over the next 4 h. The numbers of fly landings and ovipositions were recorded in consecutive 15-min periods. Four replications for each fly age were carried out for both normal and irradiated flies. Twenty irradiated females were dissected from each test using a stereomicroscope to determine the number of mature eggs in the ovaries.

Field Quality Control Tests. The survival and mating ability of T1 flies were measured under seminatural conditions. For both tests, a field cage (3.5 m high by 3 m diameter; see McInnis et al. 1996) was set over a rooted guava tree (survival tests) or artificial potted plant (mating tests) 2–2.5 m high (Silkwood Industries, Honolulu, HI). In the case of fly survival, tests were run for 7 d using 100 males of both the T1 and a recently colonized (<10 generations in the laboratory) 'wildish' strain of melon flies reared in the laboratory on zucchini. Food and water were provided in two Petri dishes of standard (3:1 sugar: protein) adult food and two water cups (200 ml each) with cotton wicks. Flies were marked with a dot of a different colored acrylic paint on the mesonotum to identify the strain. Flies were released into a field cage in the early afternoon on a given day, and all survivors were captured 7 d later. The number and proportion of surviving males per strain was recorded. Five replications were carried out. Weather data (temperature, humidity) were recorded continuously on a field Hobo unit (Pro Series). Weather data indicated that daily temperatures and relative humidities ranged between 24–32°C and 40–90%, respectively. Very little rainfall fell at the study site during these tests.

The following three types of mating tests were performed: protein level tests, fly exposure tests with cuelure, and bisexual versus male-only tests. Flies were sterilized ≈2 d before emergence, separated by sex in a cold knockdown room between 1 and 3 d of age as virgins, and held under ambient conditions until mature sexually (10–14 d old for laboratory T1 flies and 25–45 d for wild flies). For the protein level tests, irradiated T1 laboratory strain males were fed, from the time they emerged until the mating test, either the standard 3:1 (sugar:protein) diet, a lower protein diet, 6:1 (sugar:protein), or a diet of sugar (sucrose) only. Flies were marked as described above. Either laboratory males (and females, if tested) or wild males, in alternating tests, were marked to distinguish each strain.

The possibility of improving the mating ability of T1 melon flies was investigated by exposing males to the standard attractant, cuelure, in hopes of inducing a similar response shown by the oriental fruit fly to the chemical attractant, methyl eugenol (Shelly et al. 2000). On the day before cage testing, laboratory strain males (25/350-ml plastic cup) were exposed to cuelure (2 ml applied to blotter paper suspended by wire inside the cup). After the 4-h exposure period, the blotter paper was removed. Exposed males were treated in a separate room to avoid contaminating nonexposed flies. In the case of bisexual versus male-only mating tests, the sexes were maintained in separate cubical cages, in the same room under standard laboratory conditions, until their release into the field cages.

In the late afternoon of a test day, test flies were released into two field cages outside the Honolulu laboratory. Each cage contained an artificial tree, *Ficus benjamina* L. (2.2 m high with ≈700 green, plastic leaves). One hundred wild flies per sex were always used as the control population in a cage. The test population consisted of 100 sterile T1 males; in those tests involving bisexual releases, 100 sterile females

Table 1. Rearing parameters for small-scale production of the melon fly T1 genetic sexing strain, *B. cucurbitae*, in Hawaii (2001–2002)

Percent egg hatch[a] [mean (range)]	Larval collection (d)	Pupal yield (% of total)	Percent brown (male) pupae[a] [mean (range)]	Average adult emergence[b] [% (± SE)]		Average flight ability[b] [% (± SE)]	
				25–27°C	20°C	25–27°C	20°C
41.6 (40.1–44.6)	1	21.5	62.5 (55.0–80.0)	91.5	92.0	60.9	66.2
	2	38.2	47.6 (41.4–51.6)	(1.38)	(0.82)	(2.40)	(2.07)
	3	27.4	48.0 (40.7–55.5)	ns		ns	
	4	12.9	42.8 (32.3–48.3)				
	Total	100.0	50.3				

[a] Means based on seven replications.
[b] Means based on seven replications and compared using Tukey's HSD test.
ns, nonsignificant.

were also released. Flies were released into the cages in the late afternoon and allowed to settle on the leaves of the artificial tree or on the screen mesh of the cage. Approximately 30–60 min before sunset, the flies began to mate. Mating pairs were collected in small plastic vials. Male and female types were recorded along with the time and location (tree or screen) of the couple. Pairs were collected for 1–2 h until 30–60 min after sunset. For each cage, the proportion of sterile male matings out of the total number of wild female matings was calculated as the relative sterility index (RSI) (McInnis et al. 1996). Five replications each of the protein level and cuelure exposure tests and six replications of the sex-ratio tests were performed.

Statistical Analysis. Data expressed as a proportion were transformed to arc sine square root of the proportion to normalize the data. Comparisons of means were made using Tukey's honestly significant difference (HSD) tests for paired comparisons after an analysis of variance (ANOVA). The statistical software used was SAS, version 8 (SAS Institute 1998). Data in tables are presented untransformed.

Results and Discussion

Production of the T1 melon fly strain increased steadily during 2001 until reaching ≈100,000 pupae/wk by October. At this time, mass-rearing was transferred to a much larger rearing unit at the laboratory, while a small stock maintenance colony was purified, as needed each generation, and maintained. Mass production parameters are presented in Table 1. Based on 7 successive wk of production in which eggs were collected from the adult fly cages over one 24-h period/wk, egg hatch rates averaged 41.6%, with a range of 40.1–44.6%. These values are relatively low and may indicate that the sperm products resulting from adjacent segregation of meiosis are all inviable and die early as embryos (Robinson et al. 1999). Normally, about one-half of the meiotic adjacent segregation products (those carrying duplications of the autosome, rather than deficiencies) can survive into the larval or pupal stages. Because these individuals usually do not survive to produce viable adults, their presence in a mass-rearing program is a disadvantage, especially if they survive to pupate and require machine sorting and further handling. Therefore, early elimination of such adjacent segregation individuals is desirable. Another possibility for the low egg hatch rate may be that a double translocation is present in the strain. If so, egg hatch rates would normally be expected to be lower for the combination of two single translocations (Dobzhansky 1970); however, exceptions to this "rule" are known (Robinson 1976). To date, the possibility of a double translocation has not been investigated through cytogenetic evidence. However, more recently, egg hatch rates have averaged between 50 and 60% as a result of improved handling to avoid egg desiccation (D.O.M., unpublished data). This suggests a more likely scenario of a single translocation with moderate to high embryonic mortality of adjacent segregation products.

Larvae of the strain pupated over a 4-d period, with the second collection day normally the largest (Table 1). Males tended to pupate sooner than females with, on average, 62% males of the first day's collection, whereas only ≈43% of the last day's collection. This difference is significant because the percentage of males from the first day did not overlap with the percentage of males from the second, third, or fourth days (over seven replications). Adult emergence averaged >90%, with no significant difference between values for pupae held at ambient temperature (25–27°C) or at 20°C. Flight ability from 20-cm tubes averaged 60.9 and 66.2% for ambient and 20°C conditions, respectively, with no significant difference between them.

The phenotypic and genetic stability of the T1 sexing strain under low-level mass-rearing was very high (Table 2). For brown pupae, <1 female/10,000 adults scored were recovered, and of the six phenotypic contaminants, none proved to be genetically recombinant (brown) after a standard genetic testcross to white males. Therefore, it is assumed that there was some initial visual error in sorting the pupae by color. For white pupae, <1 male/100,000 scored adults were recovered, and of the 13 phenotypic contaminants, only 1 proved to be genetically recombinant (white). Presumably, the remaining 12 individuals came from either visual pupal sorting errors or perhaps were white because of nutritional deficiency.

Table 2. Observed (phenotypic) contamination and actual genetic recombination rates for brown and white pupae of the T1 melon fly sexing strain

Pupal color	No. adults obtained		Phenotype contamination rate	Genetic recombination rate
	♂♂	♀♀		
Brown	80,700	6[a]	6/80,706 = 0.000074	0/80,706 = 0.000
White	13[b]	139,500	13/139,500 = 0.000093	1/139,500 = 0.000007

[a] All test crosses (brown females and white males) yielded all white progeny (> 10 pupae) (i.e., female is genetically white and non-recombinant).

[b] Only 1 of 13 test crosses (white males times white females) yielded all white progeny (>10 pupae); the other 12 crosses yielded ½ brown: ½ white pupae (i.e., male is genetically brown and non-recombinant).

Results of varying pupal age at the time of irradiation on egg fecundity and fertility for the T1 melon fly strain are shown in Table 3. For unirradiated flies, ≈40 and 20% of the eggs hatched in the sample of 2,000 (from 50 females) for the 10- and 17-d ages, respectively. These two egg hatch values were significantly different ($P < 0.01$) by Tukey's HSD test. The large hatch drop at 17 d of age is surprising, because that is an optimum age for relatively high egg hatch in the large mass-rearing cages. This result may be because of the small number of flies and relatively low fly density in the laboratory cubical cages (50/sex), leading to a lower level of mating and egg hatch. The next three treatments (2–4) involved normal females crossed with −1, −2, and −3 males, respectively. As can be noted, the sample egg number was maximal (2,000) for each fly age, and as expected, the egg hatch rate dropped drastically in all three cases. The hatch rates were all significantly less than the first treatment ($P < 0.01$ by Tukey's HSD test) for both 10 and 17 d. The following six treatments all had females irradiated 1, 2, or 3 d before emergence, whereas the males were either normal (5–7) or irradiated 1, 2, or 3 d before emergence (8–10, respectively). Few eggs were oviposited (<12 on average), and percent egg hatch was very low (zero for most of the treatments).

These data indicate that the previously established standard melon fly irradiation dose we used, 100 Gy, is adequate to ensure high sterility for released flies in any planned SIT program with the T1 melon fly strain. For the rare irradiated T1 females released (≪0.1% under present pupal sex-sorting efficiency), mating with released T1 males will result in no (or extremely low) egg hatch (treatments 8–10). In the very unlikely event that a released T1 female mates with a fertile wild male (treatments 5–7), very few eggs with very low fertility would result. Finally, in the desired case of T1 sterile males mating with wild females (treatments 2–4), egg fecundity and fertility is not expected to be significantly higher for the −1 males compared with the −2 or −3 males (egg hatch rate <0.12% in all cases).

The effects of irradiation and female age on sterile female oviposition into host fruit by the T1 strain are noted in Table 4. For flies tested at 1.5, 2.5, and 3.5 wk of age, irradiated females landed on and oviposited in ripe zucchini fruit significantly less often than did normal females of the same age ($P < 0.01$). Pairwise

Table 3. Effect of pupal age at time of irradiation on resulting egg fecundity and fertility for the melon fly T1 genetic sexing strain

	Type of mating[a] ♂ × ♀	Fly age (d)	Total eggs [mean ± (SE)]	Egg hatch		Egg hatch rank[c]
				[Average no. (SE)]	[Percent (% range[b])]	
1.	N × N	10	2,000 (0)	808.8 (76.7)	40.4 (30.8–49.8)	a
		17	2,000 (0)	410.6 (84.3)	20.6 (14.2–29.0)	b
2.	−1 × N	10	2,000 (0)	2.2 (2.0)	0.11 (0–0.50)	c
		17	2,000 (0)	2.4 (1.2)	0.12 (0–0.30)	c
3.	−2 × N	10	2,000 (0)	0.6 (0.4)	0.03 (0–0.10)	c
		17	2,000 (0)	1.8 (1.6)	0.09 (0–0.40)	c
4.	−3 × N	10	2,000 (0)	0.6 (0.4)	0.03 (0–0.10)	c
		17	2,000 (0)	0.4 (0.3)	0.02 (0–0.05)	c
5.	N × −1	10	11.6 (3.3)	0 (—)	0 (—)	c
		17	9.2 (3.5)	0.8 (0.8)	4.71 (0–23.5)	c
6.	N × −2	10	6.8 (1.9)	0.2 (0.2)	2.86 (0–14.3)	c
		17	4.6 (3.6)	0 (—)	0 (—)	c
7.	N × −3	10	1.2 (1.2)	0 (—)	0 (—)	c
		17	4.2 (1.6)	0 (—)	0 (—)	c
8–10.	−1 × −1, −2 × −2, −3 × −3	10	2.4–6.8	0 (—)	0 (—)	c
		17	5.0–8.4	0 (—)	0 (—)	c

[a] N × N, normal ♂ × normal ♀; N × −1, normal ♂ × irrad ♀ 1 d before emergence; N × −2, normal ♂ × irrad ♀ 2 d before emergence; etc. Irradiation dose, 100 Gy.

[b] $n = 5$ replications, with up to 2,000 eggs per replication.

[c] Egg hatch percentage means associated with the same letter are not significantly different ($P = 0.01$ level) by Tukey's HSD test.

Table 4. The effects of irradiation and female age on sterile female oviposition in fruit for the melon fly T1 genetic sexing strain

Age	Reps	Irradiated T1 strain[b]		Nonirradiated T1 strain	
		Total fly landings (Average ± SE)	Total ovipositions (Average ± SE)	Total fly landings (Average ± SE)	Total ovipositions (Average ± SE)
I. 1.5 wk	4	55 (10.8)[a] a ($P = 0.007$)[c]	2.5 (1.6) b ($P = 0.008$)[c]	238.3 (18.5) A	98.5 (13.9) A
II. 2.5 wk	4	42 (12.1) a ($P = 0.0001$)[c]	3.8 (2.4) b ($P = 0.001$)[c]	332.8 (5.6) B	172.8 (6.8) B
III. 3.5 wk	4	90 (19.4) a ($P = 0.0026$)[c]	10.5 (4.1) b ($P = 0.029$)[c]	320.3 (31.7) B	166.8 (20.8) B

[a] Means (± SE) in the same row above the same letter for the same character (fly landings or ovipositions), were not significantly different ($P = 0.01$ level) by Tukey's paired *t*-test.
[b] Pupae irradiated at 100 Gy, 2 d before emergence.
[c] *P* value from Tukey's paired *t*-test for irradiated versus nonirradiated females for total fly landings or ovipositions.

comparisons of the numbers of landings or ovipositions by irradiated females at the three different ages were all nonsignificant ($P > 0.05$), whereas for nonirradiated females, the youngest age had significantly fewer landings and ovipositions than the older two ages ($P = 0.022$ and 0.004, respectively).

For the outdoor field cage survival tests, T1 strain males survived equally compared with "wildish" males. Over five replications, at least 80 T1 males (89.6 ± 3.1 SE) and 85 wildish males (87.2 ± 2.4) survived (out of 100) 7 d in the outdoor field cages. There was no significant difference in survival between the strains over the week-long period ($P = 0.635$). These survival abilities are relatively high compared with *C. capitata* under identical field cage conditions (McInnis et al. 2002), whereas only about one-half of male flies survived 2 d, and only about one-third survived 4 d. The T1 strain, therefore, seems to be a hardy strain suitable for field release.

Mating cage test results, in which the amount of sugar present in adult food was varied or where some T1 males were exposed to the male attractant, cuelure, are shown in Table 5. T1 males fed only sugar as adults did not mate at all with wildish females under field cage conditions. This dramatic result for sugar-only fed males contrasts sharply with the medfly, in which normal mating can take place for sugar-only fed males under similar conditions (El Hakim and Basilly 1986, Shelly and McInnis 2003). For the case of low protein (6:1 sugar:protein) fed to the T1 males, 13–58% of T1 males mated with the wildish females. The RSI averaged 36% for the 6:1 sugar:protein test population. For the normal protein level of 3:1 (sugar:protein), average RSI values were not significantly higher compared with the 6:1 test case ($P = 0.11$, Tukey's paired *t*-test).

Regarding the exposure of cuelure to T1 males before cage mating tests, the results in Table 5 indicate that there was no significant effect of the exposure in the five replications performed. Cuelure-exposed males mated no better with wild females than did control unexposed males, with RSIs of 0.51 and 0.52, respectively. Our results contrast with the improved mating results, after cuelure exposure, obtained in an earlier study by Shelly and Villalobos (1995). In that study, males exposed to cuelure enjoyed a mating advantage in trials conducted 1 d after exposure but not in trials conducted 3 or 7 d after exposure. Those results were also confirmed in a later study using raspberry ketone, the hydrolysis product of cuelure (Shelly 2000). Raspberry ketone is known to be sequestered by male melon flies in their rectal glands and is likely used in the production of male sex pheromone (Nishida et al. 1993). The difference in our results obtained 1 d after exposure of cuelure may be because (1) the earlier studies were conducted in the laboratory in small cages, whereas this study was conducted in much larger, outdoor field cages; and/or (2) the treated flies in the earlier studies were wildish flies (<10 generations in the laboratory) compared with

Table 5. Effects of adult diet and exposure to the male attractant, cuelure, on fly mating ability of the T1 sexing strain in outdoor field cages

Treatment	Mating type (no. pairs)		RSI[b] [mean ± (SE)]
	S × W[a] [mean ± (SE)][c]	W × W [mean ± (SE)]	
I. Sugar-only	0.0 (0.0) b	45.2 (4.3) a	0.0
II. Low protein (6:1; sugar:protein)	17.8 (5.2) a	30.0 (3.1) b	0.36
III. Moderate protein (3:1; sugar:protein)	27.2 (3.4) a	23.8 (3.7) b	0.52

[a] S × W, sterile T1 ♂ × wildish ♀; W × W, wildish ♂ × wildish ♀.
[b] RSI = relative sterility index, i.e., (S × W)/[(S × W) + (W × W)].
[c] $N = 5$ replications per treatment (mean ± SE). Means followed by the same letter in the same column are not significantly different by Tukey's paired *t*-test at the $P = 0.01$ level.

Table 6. Relative mating competitiveness of bisexual (males and females) and male-only sterile populations of the T1 melon fly genetic sexing strain against wild melon flies in field cages

Treatment	Replications	Mating type (average no. pairs)					RSI[b]
		S × S[a]	S × W	W × S	W × W	Total	
I. Bisexual (♂ + ♀) sterile T1	6	36.3	28.0	18.0	46.3	128.6	0.37
II. Male-only sterile T1	6	S × W		W × W			
		43.5		34.8		78.3	0.56

[a] S × S, sterile ♂ × sterile ♀; S × W, sterile ♂ × sterile ♀, etc.

[b] RSI = relative sterility index, the proportion of all wild female matings made by sterile males, i.e., (S × W) / [(S × W) + (W × W)]. RSI values transformed to normalize before mean comparison by Tukey's paired *t*-test ($P = 0.00031$, for means of bisex [0.37] versus males-only [0.56].

flies from a laboratory-adapted strain well over 10 yr old in this study.

Male-only sterile populations of the T1 sexing strain outcompeted bisexual sterile populations against wild flies in field cages (Table 6). In the case of bisexual releases of sterile T1 flies over six replications, the RSI averaged 0.37. This value indicated that about twice as many wild females mated with wild males compared with laboratory sterile males. For the case of male-only T1 tests, the RSI averaged 0.56. This value suggests that the absence of T1 sterile females resulted in an approximately two-fold increase in the relative mating success of T1 males competing with wild males for wild females (≈1:2 to ≈1:1). The difference between bisexual and male-only RSI values was highly significant ($P = 0.00031$). The finding of a significant mating advantage for male-only sterile melon populations compared with bisexual populations was expected based on open field SIT test results from the medfly (McInnis et al. 1994, Rendon et al. 2000). Previously, however, mating cage tests showed no significant mating advantage for medfly male-only test populations in Hawaii, Guatemala, and Chile (McInnis et al. 1997). Another *Bactrocera* species, the oriental fruit fly, *B. dorsalis* (Hendel), showed an advantage for male-only field cage populations compared with bisexual populations in mating trials against its sibling species, *B. carambola*, in Suriname, South America (McInnis et al. 1999). In that case, the absence of *B. dorsalis* females allowed conspecific males to cross-mate with *B. carambola* females.

The above collection of laboratory and field cage studies indicates that the T1 sexing strain can be reared with high offspring rearing qualities, high survival ability in outdoor cages, and high mating competitiveness, especially when in male-only sterile fly releases. All of these factors suggest that the strain would be a good candidate for sterile fruit fly programs against the melon fly. Of course, the relatively higher cost of producing and releasing this genetic sexing strain needs to be factored into the overall cost–benefit equation. A male-only melon fly SIT project has successfully been carried out in the USDA Area-Wide fruit fly program in Hawaii over the last 2 yr (2002–2003) and will be reported on in the near future.

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